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A Double-Blind, Placebo Controlled Study to Evaluate the Safety and Immunogenicity of the New, Live, Oral Type-4 and Type-7 Adenovirus Vaccines in Military Trainees

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Background: Adenoviruses have been an important cause of febrile acute respiratory disease (ARD) in military recruits since they were first implicated as a cause of ARD in 1953 in an epidemic at Fort Leonard Wood, MO[1]. Since then, adenovirus types 4 and 7 have been the most important cause of ARD among military recruits in the United States[2], resulting in significant morbidity, loss of training time, and in rare instances, mortality[3]. Prior to the introduction of live, oral adenovirus vaccines in 1971, adenoviral infections caused the hospitalizations of 10% of military recruits, 90% of pneumonia hospitalizations, and more than 67% of all respiratory diseases in basic training[4-7]. Outbreaks in the civilian population, though rare, have occurred[8,9].

In the 1970s, the Department of Defense (DoD) contracted Wyeth Laboratories to produce oral, live, enteric coated vaccines against adenovirus serotypes 4 and 7. These Wyeth vaccines were unique in that they produced selective, asymptomatic infection of the gastrointestinal tract[11], followed by the production of protective antibody, without producing a systemic infection, or viral cross interference[11]. These vaccines were used extensively in military basic training centers from 1971 until 1996. They were safe and effective vaccines and their use resulted in the virtual elimination of adenovirus-associated ARD[11-17] in basic training centers. In 1996, Wyeth discontinued production of the vaccines. When the stockpile of vaccines was exhausted in 1999, adenovirus-associated ARD returned to pre-vaccine levels[6,18-21]. During the time period from 1997 to 2000, after the previously licensed vaccines were discontinued, there were three significant adenovirus outbreaks among US Army basic trainees. Between 23 April and 6 May 2000, one such outbreak of adenovirus type 4 ARD resulted in an ARD admission rate of 2.9% per week; the overall admission rate for one training company was 58.6%[10].

As an illustration of the effect of vaccination on the rates of adenovirus ARD in this population, the outbreak at Fort Jackson, SC from May through December 1997 is illustrative[22]. During this time period, there were 1,018 basic trainees with febrile ARD admitted to the post hospital. Of these, 673 (66.1%) were positive for adenovirus type 4 (isolation rates of over 90 percent were seen by the end of the outbreak). At the peak of the epidemic, 70 soldiers per week were hospitalized which translated to a hospitalization rate of 1 per 100 soldiers on post. When the adenovirus vaccination was reintroduced in November 1997, the rates of febrile ARD and adenovirus type 4 isolation declined significantly.

The loss of the adenovirus vaccines and the subsequent surge of adenovirus-associated ARD prompted the DoD to seek a new manufacturer for the adenovirus vaccines. A cost-effectiveness analysis[23] revealed substantial case prevention and cost savings with reinstatement of seasonal or year-round vaccination. Duramed Research (a division of Barr Laboratories, Inc.) was contracted by the DoD to produce and license the new ADV-4 and ADV-7 vaccines. This study summarizes the results of a Phase 1 study of the new oral, live, enteric-coated adenovirus vaccines produced by Duramed.

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Methods: The primary objective of the Phase 1 study was to evaluate the safety of the adenovirus type 4 and type 7 vaccines administered together compared with placebo. Secondary objectives included the evaluation of the immune response of the vaccines at Day 28 following vaccination, and characterization of the duration of vaccine virus shedding. The vaccines were manufactured by Duramed Research using virus grown from the virus seeds provided by Wyeth Laboratories, the previous manufacturer. Each enteric coated tablet contained at least 32,000 tissue culture infectious dose ($TCID_{50}$) of ADV-4 or ADV-7. The protocol was approved by the WRAIR and BAMC institutional review boards and the Surgeon General's Human Subjects Research and Review Board. Volunteers were recruited from the Combat Medic Training Course (91W) at the US Army Medical Department (AMEDD) Center and School, Fort Sam Houston, TX. Those found to be without detectable anti-ADV-4 and/or anti-ADV-7 neutralizing antibodies were randomly assigned in a 1:1 ratio to receive either the vaccine tablets or placebo tablets. Vaccine safety was assessed by a daily symptom diary card completed by the volunteers during the first week after vaccination, review of adverse events (AE) at follow up visits at days 7, 14, 21, 28 and 56 days after vaccination and surveillance review of clinic visits and hospital admissions. Serum neutralizing (SN) antibodies to ADV-4 and ADV-7 were assessed using the colorimetric microneutralization method by the Department of Virus Diseases, WRAIR. The presence of vaccine virus in blood, stool and throat was determined by the Department of Pathology, Walter Reed Army Medical Center (WRAMC), by inoculation of A549 cells. Specimens demonstrating cytopathic effect were stained by direct immunofluorescent technique (D3 DFA). Specimens positive by IFA were tested by polymerase chain reaction (PCR) assays by the Naval Health Research Center (NHRC) to identify the presence of ADV-4 and/or ADV-7. If ADV-4 was identified in throat specimens, wild-type ADV-4 and vaccine virus were distinguished by additional PCR assays performed at NHRC.

Results: A total of 407 soldiers volunteered to enroll and underwent screening for determination of eligibility. Of these, 72 (18%) and 74 (18%) were seronegative for ADV type-4 and ADV type-7, respectively. This result was consistent with a seroprevalence study carried out by WRAIR investigators 5 months before the trial in the same population (91W), which showed an ADV-4 seronegative rate of 11% and ADV-7 seronegative rate of 22%. Fifty-eight volunteers met all eligibility criteria and were vaccinated with either the adenovirus vaccines (30) or placebo (28). Fifty-four volunteers completed the study. None of the volunteers dropped out for issues related to vaccine safety. In general, all AEs were of a minor nature and did not interfere with training. The most commonly reported AEs in the vaccine and placebo recipients were nasal congestion (33% and 57%, respectively), cough (33% and 36%, respectively), sore throat (27% and 29%, respectively), headache (20% and 21%, respectively), abdominal pain (17% and 4%, respectively), arthralgia (13% and 0%, respectively), nausea (13% and 7%, respectively) and diarrhea (13% and 21%, respectively). Among vaccine recipients, virus was shed in the stools of 73% and 70% of volunteers seronegative to ADV-4 and ADV-7, respectively. Vaccine virus was not recovered from the stool of placebo recipients. All vaccinated subjects who seroconverted also shed virus in their stools. Vaccine virus shedding was first detected at 7 days after vaccination and not detected after 21 days. Adenovirus was not recovered from the blood of any volunteer and no vaccine virus was detected in the throat secretions. Wild-type ADV-4 was isolated from the throats of 3 volunteers (all received placebo), 2 whom subsequently developed ARD. All 3 placebo subjects subsequently developed neutralizing antibodies to ADV-4. No wild-type virus was isolated from the throat of any vaccine recipient. Seventy-three percent of vaccine recipients seroconverted to ADV-4 (GMT 13.9), while 65% seroconverted to ADV-7 (GMT 14.7) by Day 28. Thirty-three percent of placebo recipients seroconverted to ADV-4 during the study, providing further evidence of the ongoing transmission of ADV-4 in this population.

Discussion: Adenovirus respiratory illness continues to be a major factor affecting the basic training of military recruits. For over 25 years, recruits received effective, oral, live, enteric-coated ADV vaccines to

prevent illness from adenovirus serotypes 4 and 7. However, existing supplies of the approved ADV-4 and ADV-7 adenovirus vaccines were exhausted and the vaccines are now no longer available. Outbreaks of adenoviral respiratory illness have reemerged in military settings with rates of clinical infection that are similar to those observed prior to the availability of the Wyeth vaccine. Rarely, there have been deaths due to adenoviral pneumonia in this population. Reinstatement of an adenovirus vaccination program is urgently needed. The Duramed ADV vaccines are designed to be equivalent to the Wyeth vaccines in both vaccine virus (cultured in WI-38 human diploid fibroblast cells) and delivery route (oral administration). The Wyeth vaccines were studied extensively and administered to several million recruits over more than 25 years. They had an excellent safety and efficacy profile. Thus, the purpose of this Phase I study was to evaluate the safety and immune responses of the Duramed vaccines against the history of the Wyeth-approved products and their proven track record.

In this study, the Duramed live oral ADV-4 and ADV-7 vaccines were safe and well tolerated. The overall incidence rates of adverse events were similar between the vaccine and placebo groups. The vaccine group experienced less nasal congestion, less pneumonia, less fever and less WBC elevations than the placebo group. There was circulating adenovirus type-4 among this population during the study, as evidenced by the 2 subjects (placebo recipients) who developed pneumonia caused by wild-type ADV-4, confirmed by throat swab culture results. There were no vaccine recipients who developed pneumonia due to wild-type ADV-4. However, more abdominal pain and diarrhea were reported in the vaccine group. This could be related to oral administration route of the vaccines. The abdominal pain and diarrhea only appeared within the first week after vaccination, was transient and was mild to moderate in intensity. This is consistent with the safety profile reported in the product labeling of the prior approved Wyeth products. No soldiers missed any training time as a result of taking these vaccines. The vaccine virus was not detected in any throat cultures and sera. No one discontinued the study due to SAEs. No deaths occurred.

It should also be noted that the Duramed ADV vaccines induced an asymptomatic ADV infection in the gastrointestinal tract in previously seronegative individuals, as was previously found for the Wyeth vaccines. A marker for vaccine virus gastrointestinal replication is isolation of ADV in the stool. Administration of the Wyeth vaccines had consistently resulted in ADV being detected in stool samples collected up to 28 days or longer after vaccination. In the Phase 1 study, the Duramed vaccines demonstrated a similar pattern of gastrointestinal infection as evidenced by isolation of ADV in stool samples between days 7 to 21. Fecal viral shedding data showed that after vaccination, among those who had negative pre-immunization antibodies, seroconversion was associated with fecal viral shedding; all subjects who had vaccine virus in stools seroconverted (9 in ADV-4 group and 12 in ADV-7 group). Interestingly, not all who exhibited a boost in titer excreted vaccine virus in stools. In previous studies, vaccine virus shedding in the stools was noted as far out as 39 days after vaccination[10]. Under conditions found in military training, the risk of vaccine virus transmission from vaccinees to non-vaccinees was felt to be remote[13,24,25] because of the lack of intimate contact between recruits "of the type" that exist within families. The risk of spread would increase with intimate contact between persons, as was found in earlier studies[26,27]. These findings suggest that the Duramed formulations are functioning in the same fashion as the Wyeth ADV vaccines – the vaccines selectively infect the lower intestinal tract and bypass the upper respiratory tract. Thus, it can be stated that the Phase 1 clinical data suggest comparable performance of the Duramed vaccines to the Wyeth vaccines.

Both vaccines were associated with substantial rates of seroconversion. As described in the protocol and statistical analysis plan for the primary efficacy evaluation of the observed rate of seroconversion at Day 28, the seroconversion rate was 72.7% (8 out of 11) for the ADV-4 vaccine group versus 30.0% (3 out of 10) for the ADV-4 placebo group. The seroconversion rate for the ADV-7 vaccine group was 64.7% (11 out of 17) versus 0 % (0 out of 14) for the placebo group. Denominators are the total number of subjects per group that were seronegative to ADV-4 or ADV-7 at Day 0. Since seroconversion by a certain timepoint is a more clinically relevant outcome, it is important to note that 9/11 (81.8%) subjects in the vaccine group and 3/10 (30%) in the placebo group seroconverted for ADV-4 by Day 56; 12/17 (70.6%) subjects in the vaccine group

and 0/14 (0.0%) in the placebo group seroconverted for ADV-7 by Day 56. Seroconversion in the ADV-4 placebo group was attributable to ADV-4 wild type infection as wild-type ADV-4 was isolated from the throats of all three of the volunteers (placebo recipients), including 2 who presented with ARD. The peak incidence of ARD varies among different basic training centers; peaks vary from week 3 to as far as week 6-7. In this study, it appears the majority of vaccine recipients will seroconvert by day 28 and be protected against disease; however, efficacy may not be accurately reflected by seroconversion alone at this time point; indeed, it may underestimate it in this case, as it is known that in similar populations, persons who do not have neutralizing antibody are in fact, protected[28-30]. True vaccine efficacy can only be established in an efficacy trial.

For those subjects who were seropositive to either ADV-4 or ADV-7 at baseline (Day 0), 4/19 (21.1%) in the ADV-4 vaccine group and 0/18 (0.0%) in the ADV-4 placebo group showed a booster effect by Day 56. Nine of 13 subjects (69.2%) in the ADV-7 vaccine group and 1/14 (7.1%) in the ADV-7 placebo group, whose ADV-7 titer went from 1:583 at baseline to 1:5210 at Day 56, showed ADV-7 booster effects by Day 56. Denominators are the number of subjects ADV-4 or ADV-7 seropositive at Day 0. Among those seropositive to ADV-4 at the beginning of the study and who showed a booster effect, none shed ADV-4 virus at any time during the study. Six out of 9 volunteers who received ADV-7 vaccine and showed a booster effect excreted ADV-7 virus, none detected after 14 days. This has been observed in the past (L. Binn, unpublished results) and may represent asymptomatic infection of the GI tract in persons who have antibody at low titers (L. Binn, personal communication).

The ADV-7 boost in the placebo recipient above could be due to a potential ADV-7 wild type infection, but the lack of circulating ADV-7 disease in this population and the lack of a positive throat swab for ADV-7 in this subject refutes this assumption. This volunteer seroconverted to ADV-4, excreted ADV-4 in the stool, and no ADV-7 was found in the stool. More likely, this reflects a phenomenon known as the heterotypic antibody response, as exposure to a homotypic antigen results in the development of heterotypic antibodies to the antigen. This is a phenomenon observed in several viral infections, including adenovirus[1, 31-34]. This volunteer may have been exposed to wild-type ADV-4, developed a subclinical infection, and subsequently developed antibodies to ADV-7. It would have been unlikely due to vaccine-induced antibody, as the volunteer received placebo, and vaccine-induced antibody titers are less than titers in natural infection[10]. It is unlikely to have been due to dual ADV-4 and ADV-7 infection, due to lack of circulating ADV-7 at the time, and, to the author's knowledge, no reports of dual ADV infections exist in the literature. It may reflect a recall mechanism in which the subject was exposed to ADV-7 early in life, developed neutralizing antibodies which were undetectable by study beginning, then, when challenged with wild-type infection, expressed anti-ADV-7 antibodies[31,34].

Interestingly, 12 subjects who were ADV-4 negative at screening (36.4%) converted to seropositive by vaccination day. Their titers ranged from 4 to 273. This could represent the presence of circulating ADV-4 in the study population, manifesting as a subclinical infection. The presence of circulating wild-type ADV-4 was confirmed by the 2 placebo recipients who developed wild-type ADV-4 pneumonia (and another placebo recipient who had a positive wild-type ADV-4 throat swab on a scheduled follow up visit). All three of these placebo recipients also seroconverted to ADV-4. For ADV-7, 4 volunteers who were seropositive at screening turned seronegative at vaccination day, while 4 volunteers who were seronegative at screening seroconverted by vaccination day. The majority of these had titers around 4, so these may represent the variations in a biological assay. One subject in the latter group had a titer of 20; this could have potentially been considered a wild type ADV-7 infection (unlikely), or a heterotypic antibody response to ADV-4, given the lack of circulating ADV-7 at the time. Further, there were no documented cases of ADV-7 pneumonia during the study, no positive wild-type ADV-7 isolated from throat swab specimens, and from Day 0 to Day 56, in the placebo group, no one seroconverted for ADV-7. The overall findings suggest that the background ADV-4 infection rate for this study was around 30% and that ADV-7 infection was barely detectable, if it occurred.

Given the similarity of the Duramed and Wyeth vaccines, results of this study provide an adequate basis of information regarding safety and efficacy of the Duramed product, and support its further evaluation in a Phase III setting. In addition, the safety findings of this study are consistent with the safety profile reported in the product labeling of the approved Wyeth product. Further studies to evaluate the safety and efficacy of ADV-4 vaccine and safety and immunogenicity of ADV-7 vaccine are underway.

Conclusion: The new Duramed adenovirus vaccines were safe and induced a good immune response in the study population. This Phase 1 study suggests that oral administration of the Duramed vaccines will, like the previous Wyeth vaccines, result in an asymptomatic infection of the gastrointestinal tract and induce protective neutralizing serum antibodies. This study, and the preliminary seroprevalence study performed in 91W blood donors, also reveals that ADV disease is also an ongoing problem in advanced individual training (AIT) personnel. The full extent of this problem is uncertain. Further studies are required to better understand the disease burden and the threat posed to AIT training throughout the DoD. Based on the encouraging results of this Phase 1 trial, expanded trials for safety and efficacy of the vaccine are planned. The pivotal Phase 3 efficacy trial, required for licensure by the Food and Drug Administration, is scheduled to take place at Fort Jackson, SC and Great Lakes, IL basic combat training centers for the Army and Navy, respectively, where ADV is currently a significant ongoing threat. Successful completion of these trials and licensure of the new ADV vaccines will mark the return of a key component in the preventive armamentarium against a major threat to new recruits and will greatly contribute to the health protection of our Soldiers, Sailors, Airmen, Marines and Coast Guardsmen.

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Adenovirus Vaccine Restoration

Presentation to
25th Army Science Conference

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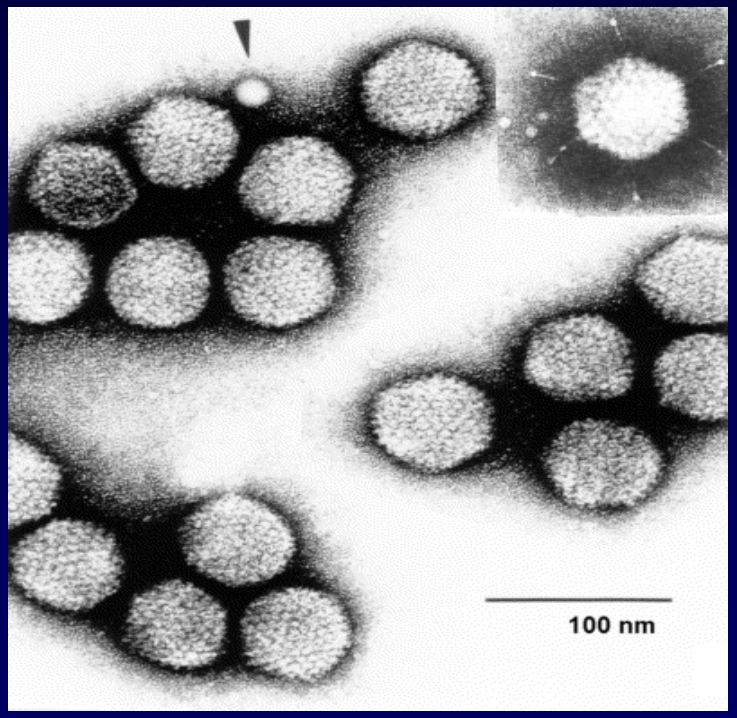
Outline

- Adenovirus Background
- Vaccine Development
- Adenovirus Vaccine Phase 1 Trial
- Future Clinical Development

Adenovirus Background

- A ubiquitous, nonenveloped, double-stranded DNA virus that causes disease in man
  - Acute respiratory disease (ARD)
    - Unique military problem
  - Eye
  - Genitourinary
  - Gastrointestinal
- Divided into several (51) serotypes
  - Types 4 and 7 account for majority of all acute respiratory diseases in military basic recruits
  - Occasionally types 3, 14 and 21

Adenovirus



Büchen-Osmond, C ICTV dB Descriptions, p2

The Disease

- Acute Respiratory Disease (ARD)
  - Fever (≥100.5ºF) plus one or more of the following: sore throat, cough, stuffy nose, runny nose
  - In severe cases, Pneumonia
  - Death
    - Six DoD recruit deaths in last five years associated with Adenovirus (two were Army recruits)

Threat

Nature of the threat:

- Debilitating illness in DoD recruits
  - Rarely seen in civilian populations
- Rapid spread from soldier to soldier
  - Aerosol route
- Most infections inapparent
  - Nearly all susceptibles infected
- No antiviral treatment available
- No vaccine currently available

Impact

Impact on Soldiers:

- Acute Respiratory Disease is pervasive in IET (especially in BCT/OSUT)
  - Longstanding problem
  - Most important cause of ARD is Adenovirus
    - Others: Flu A, Flu B, M. pneumoniae, C. pneumoniae
  - Of those IET Soldiers hospitalized for severe ARD
    - 60% to 90% result from Adenovirus
- 25% to 40% of ALL trainees will get Adenovirus-associated ARD

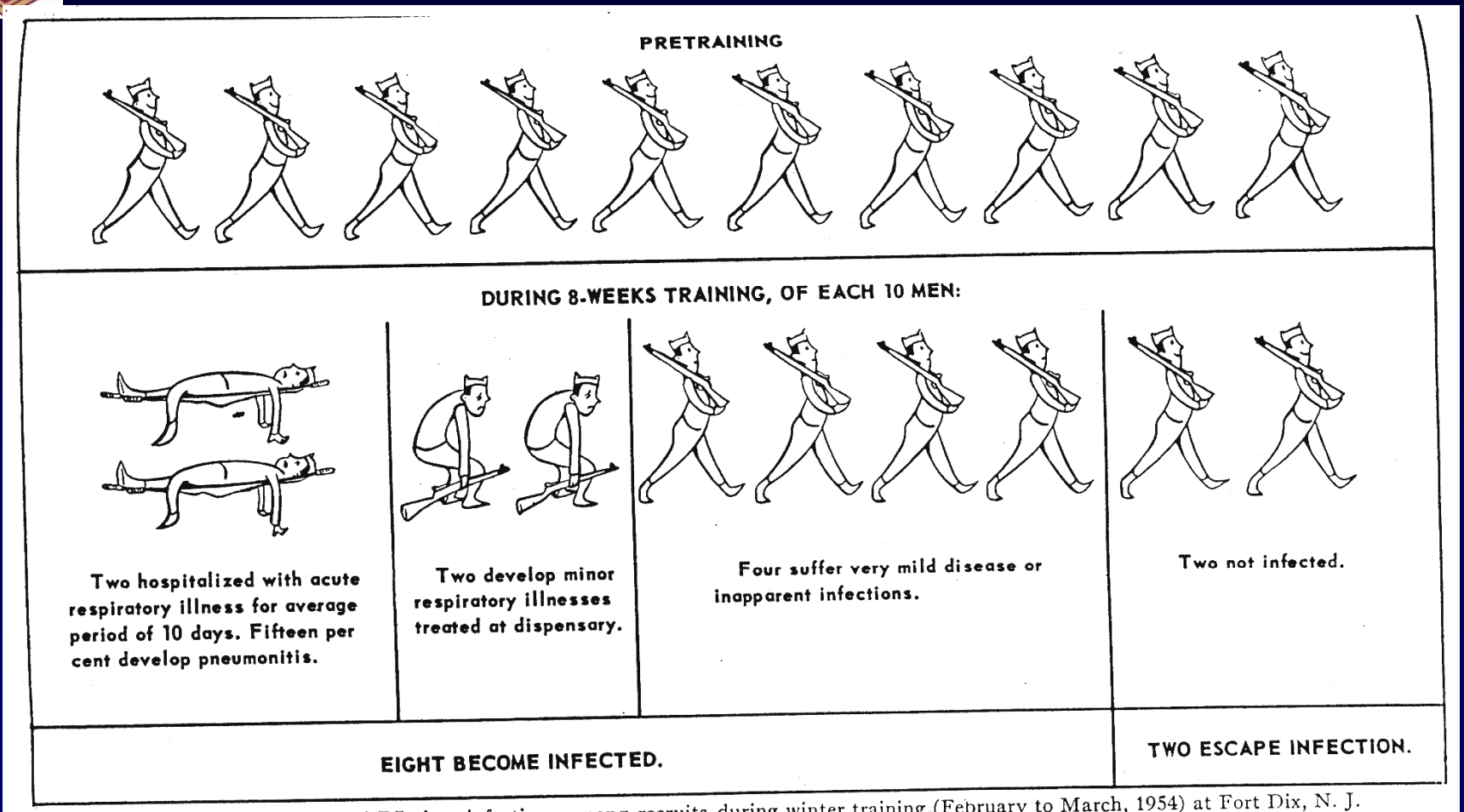


FIGURE 4. The development of RI virus infections among recruits during winter training (February to March, 1954) at Fort Dix, N. J.

Hilleman, MR Ann. NY Acad Sci, 67, 267, 1957

Impact (cont'd)

Impact on Training:

- Training time lost
- Training effectiveness reduced
- Training mission adversely affected
  - Hospitalizations
    - Increased stress on medical resources
  - Re-training (recycle)
- Training costs increased due to recycles

Disease Co-Factors

BCT environment

- High stress and reduced sleep
  - Immune system less effective
- Open Bay Barracks
  - Facilitates spread of disease
  - The lower the square footage per Soldier, the higher the incidence of ARD
  - Sleeping arrangement
    - Head-to-Head vs. Head-to-Toe

Countermeasure

- Two pills (against types 4 and 7)
  - Developed by NIH and DoD in 1960s
  - Licensed 1980
- Used in recruits year-round (1971-1996)
  - Eliminated ARD epidemics due to adenovirus
- About 27,000 DoD trainee hospitalizations were prevented in the *first year alone*

Loss of Vaccine

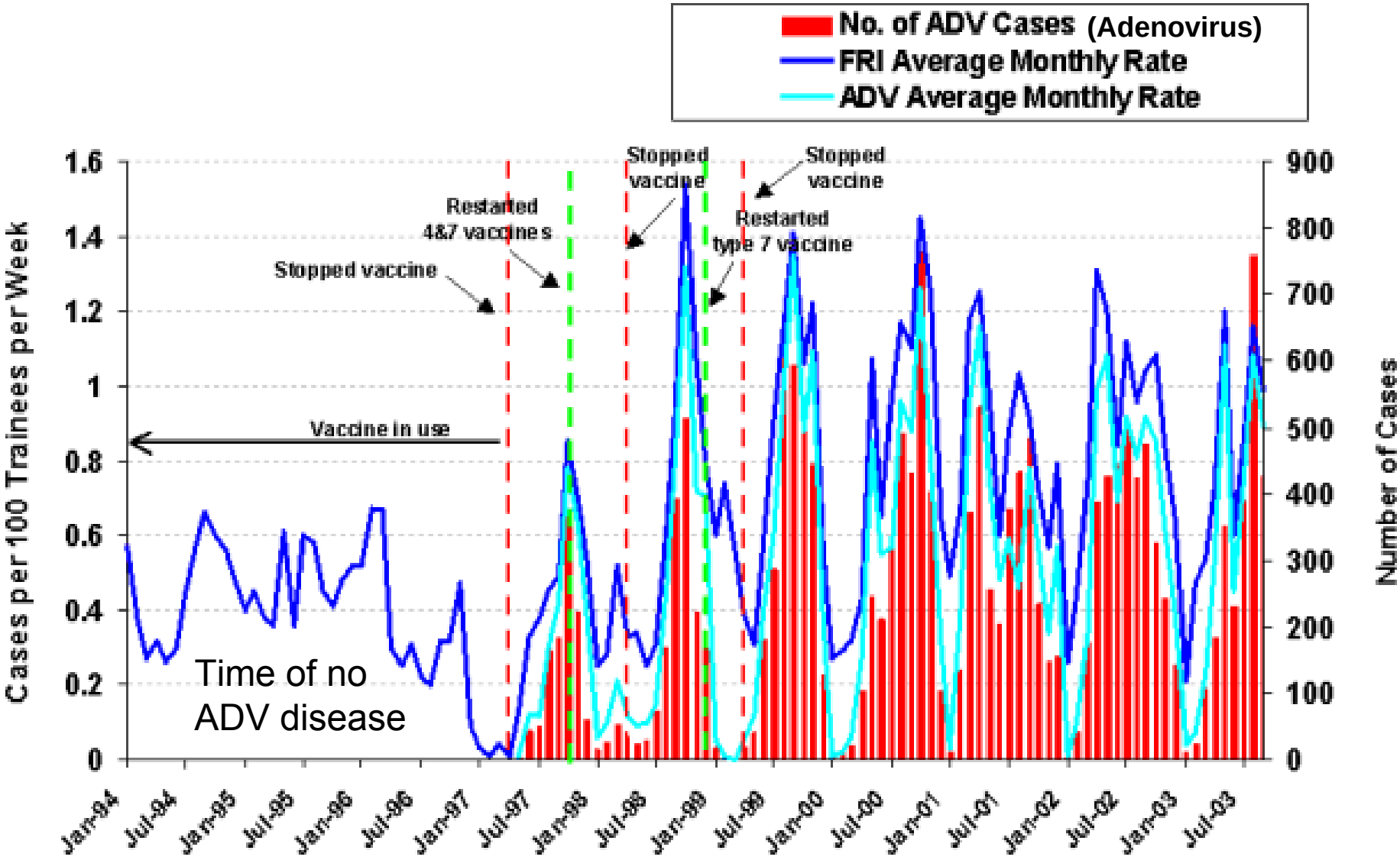
Loss of Adenovirus Vaccine:

- Manufacturer ceased production in 1996

Impact:

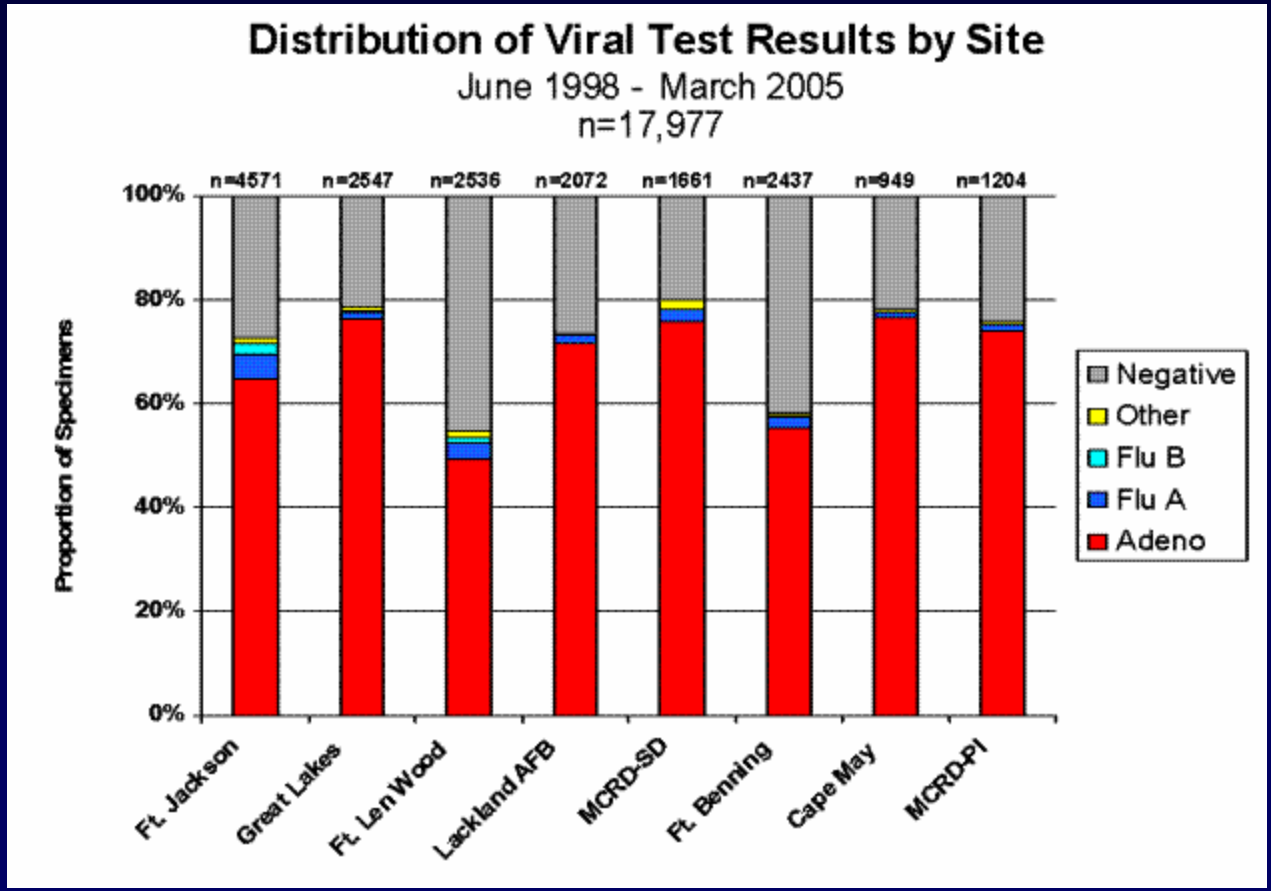
- Disease levels returned to pre-vaccine era
  - Approximately 45,000 cases annually
  - Vaccine could prevent 90% of these ARD illnesses

Fort Jackson: Febrile Respiratory Illness (FRI) and Adenovirus Disease Rates



Courtesy of CDR Kevin Russell, Naval Health Research Center

Current Adenovirus Epidemiology



Data from NHRC

Solving the Problem

- OSD-HA tasked MRMC to acquire a new vaccine against adenovirus
- Contract awarded to Barr Labs in 2001
- Barr produced new vaccine tablets and provided them to MRMC for testing in 2004

New Vaccine

First Vaccine Tablets Against Type-4 were Produced 31 Jan 2004

Photo Courtesy Dr. A. Towle

Vaccine Development

A Phase 1, Randomized, Double-Blind, Placebo Controlled Study to Evaluate The Safety And Immunogenicity Of The Live, Oral Type-4 and Type-7 Adenovirus Vaccines

Walter Reed Army Institute of Research — PI: Dr. Arthur Lyons
Brooke Army Medical Center — PI: Dr. Jenice Longfield
AMEDD Center and School
Walter Reed Army Medical Center
Naval Health Research Center
U.S. Army Medical Materiel Development Activity
Duramed Research, Inc (Barr Laboratories)

Objectives

Primary:

1. Evaluation of the safety of the Barr type 4 and type 7 oral adenovirus vaccines administered together.

Secondary:

1. Adenovirus types 4 and 7 neutralizing antibody production and titer in seronegative subjects

2. Duration of vaccine virus shedding in the stool and throat secretions in vaccine recipients.

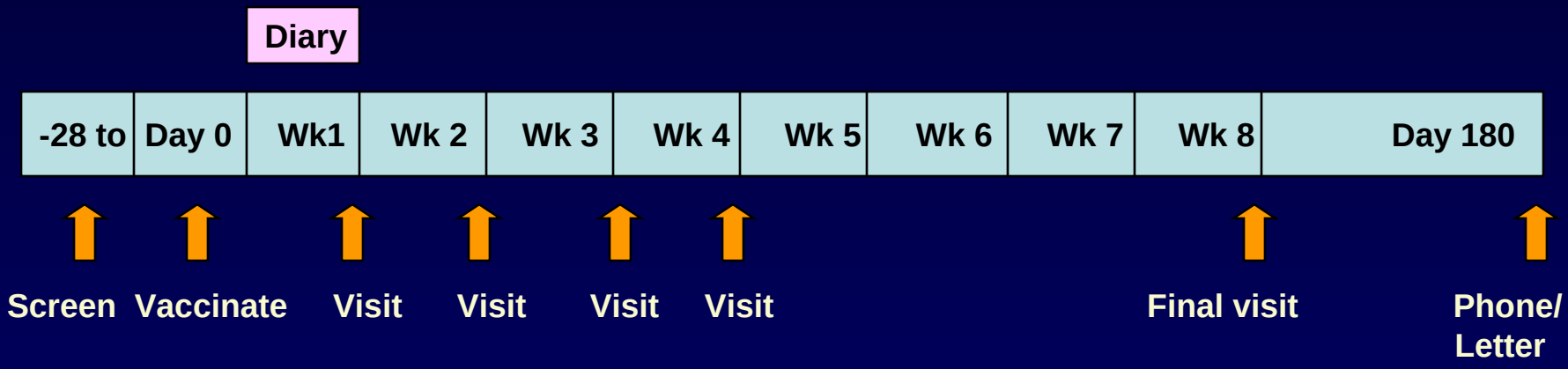
Rationale

- Military subjects to simulate BCT
  - Demographics
  - Confinement
- Minimize potential secondary spread of vaccine virus
- Low likelihood of active Adv 4 or 7 circulation
- Relative ease in recruitment
  - Large numbers available for screening

Trial Summary

- Briefed three 91W Companies
- Consented 407 for Eligibility Screening
- Vaccinated 58 Volunteers on 26 Sep 04
- Followed Closely for 56 Days
  - Adverse Event Checks (diary cards, follow up)
  - Blood, Throat, Stool Specimens, Urine pregnancy
  - ARD surveillance (TMC, BAMC ER)

Study Design



Serology: Screen, Day 0, Wks 1,2,4,8
Throat: Day 0, Wks 1,2,3,4,8
Stool or rectal swab: Day 0, Wks 1,2,3,4,8
Viremia: Day 0, Wks 1,2,4,8
All febrile ARD worked up

Inclusion/Exclusion

- Healthy 18-40 yo
- Informed Consent
- If female, not be pregnant or nursing
- Seronegative to at least one serotype (4 or 7)
- No prior enlisted military service before 1998
- No history of major medical illnesses
- No acute illness or abnormal physical exam
- No HIV, active Hep B, C
- No other vaccinations within 30 days prior to Day 0

Subject population

407 91W screened

Adv 4 (+)	Adv 7 (+)	68%
Adv 4 (-)	Adv 7 (+)	14%
Adv 4 (+)	Adv 7 (-)	14%
Adv 4 (-)	Adv 7 (-)	4%
Adv 4 seropositive		82%
Adv 7 seropositive		82%

Subject population

- 58 (14%) seronegative volunteers enrolled
  - 30 received vaccine
  - 28 received placebo
- 54 (93%) volunteers completed study
  - 4 (7%) dropped out (not vaccine related)

Change of Antibody Status During Screening Period

	Vaccine		Placebo		Total	
Antibody	Screening	Day 0	Screening	Day 0	Screening	Day 0
ADV4(-)	17	11	16	10	33	21
ADV4(+)	13	19	12	18	25	37
Total	**30**	**30**	**28**	**28**	**58**	**58**
ADV7(-)	17	17	14	14	31	31
ADV7(+)	13	13	14	14	27	27
Total	**30**	**30**	**28**	**28**	**58**	**58**

Results (AE)

- Most common adverse events reported (>10%)
  - Nasal congestion
  - Cough
  - Arthralgias
  - Nausea
  - Abdominal Pain
  - Sore Throat
  - Headache
- None differed significantly from placebo

Safety: SAE

Day 0-56

- 2 pneumonia hospitalizations (one vaccine, one placebo)
- 1 ARD hospitalization (placebo)

Day 180

- 1 hospitalization for “appendicitis” (vaccine)
- 1 hospitalization for MRSA thigh abscess (placebo)

Other Safety Parameters

- Lab (CBC, LFT, Cr)
  - No abnormalities felt to be due to vaccine
- Vital Signs
  - No abnormalities felt to be due to vaccine
- Viremia
  - No viremia
- Vaccine Virus in Throat Swabs
  - No vaccine virus detected in vaccine recipients

Immunogenicity at Day 28

	Vaccine				Placebo			
	Converted*	N**	%	95% CI***	Converted*	N**	%	95% CI***
Type 4	8	11	72.7	(39.0, 94.0)	3	9	33.3	(7.5, 70.1)
Type 7	10	16	62.5	(35.4, 84.8)	0	13	0.0	(-,-)

* Conversion is defined as at least a 4-fold increase in Ab titer in a previously seronegative (<1:4) subject to a titer ≥ 1:8

** Total # of subjects who were type 4 or 7 seronegative at Day 0 and whose D28 data not missing

***Exact binomial method

Cumulative Type-4 Seroconversion

	Vaccine (N=11)*		Placebo (N=10)*	
	Converted	%	Converted	%
Day 7	0	0.0	0	0.0
Day 14	6	54.5	2	20.0
Day 28	8	72.7	3	30.0
Day 56	9	81.8	3	30.0

*N=Total number of subjects type-4 seronegative at Day 0

Cumulative Type-7 Seroconversion

	Vaccine (N=17)*		Placebo (N=14)*	
	Converted	%	Converted	%
Day 7	0	0.0	0	0.0
Day 14	10	58.8	0	0.0
Day 28	11	64.7	0	0.0
Day 56	12	70.6	0	0.0

*N=Total number of subjects type-7 seronegative at Day 0

Cumulative Type-4 Booster Effect

	Vaccine (N=19)*		Placebo (N=18)*	
	Boosted**	%	Boosted**	%
Day 7	1	5.3	0	0.0
Day 14	4	21.1	0	0.0
Day 28	4	21.1	0	0.0
Day 56	4	21.1	0	0.0

*N=Total number of subjects type-4 seropositive at Day 0

**Booster Effect was defined as at least a 4-fold increase in Ab titer in a previously seropositive (titer ≥ 1:4) subject

Cumulative Type-7 Booster Effect

	Vaccine (N=13)*		Placebo (N=14)*	
	Boosted	%	Boosted	%
Day 7	1	7.7	0	0.0
Day 14	6	46.2	0	0.0
Day 28	8	61.5	1	7.1
Day 56	9	69.2	1	7.1

*N=Total number of subjects type-7 seropositive at Day 0

**Booster Effect was defined as at least a 4-fold increase in Ab titer in a previously seropositive (titer ≥ 1:4) subject

Viral Shedding

- Type 4
  - Ab (-): 8/11, (Day 21)
  - Ab (+): 0/19
  - Placebo: (Ab-): 2/10; (Ab+): 0/18
- Type 7
  - Ab (-): 12/17, (Day 14)
  - Ab (+): 6/13, (Day 14)
  - Placebo: (Ab-): 0/14; (Ab+): 0/14

Correlation Between Viral Shedding and Immunogenicity in the Vaccine Group

Day 0 Ab	# Vaccinated	Fecal Viral Shedding	Immunogenicity
Type 4 (-)	11	8+1 positive	9 converted
Type 4 (+)	19	0 positive	4 boosted
Type 7 (-)	17	12 positive	12 converted
Type 7 (+)	13	6 positive	9 boosted

ARD

- SAE: Pneumonia x 3
  - 2 (placebo) with wt ADV-4 in throat swab
  - 1 (vaccine) with negative throat swab
- Follow up
  - 1 (placebo) with sore throat, cough
    - wt ADV-4 in throat swab

Phase 1 Study Summary

- Adenovirus 4 and 7 vaccines
  - Performed as expected
  - No vaccine virus present in blood, throat
  - No training days lost
- Vaccine viral shedding limited to 21-28 days
  - All who shed vaccine virus seroconverted
- Immunogenicity estimated at 40-90%
- Evidence of wt Adv 4 circulation during study

Clinical Development Plan

- Next clinical trial currently underway
  - Phase 3 Safety, Immunogenicity, Manufacture consistency
  - Started 30 Sept 2006
  - Ft. Jackson, SC (USA) and Great Lakes, Ill (USN)
  - 4000 volunteers

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Additional Slides

Stages of Review and Regulation

Clinical Investigational Plan

IND

Phase 1
Safety
Immuno-
genicity

Phase 2
Immuno-
genicity
Safety
Dose
Ranging

Phase 3
Efficacy
Safety
Immuno-
genicity

BLA
(PLA)
(ELA)
Data to
support
approval;
Inspection

Phase 4
Inspection
Safety
Efficacy
Lot Release

BLA Supplement
(PLA or ELA Suppl)
Post-approval
Changes:
New Indications
Dosing
Manufacture
Equip./Facilities

Establishment of Manufacturing
and Testing Controls, Specifications

IND =Investigational New Drug Application;
BLA=Biologics License Application

Stages of Review and Regulation

Clinical Investigational Plan

IND

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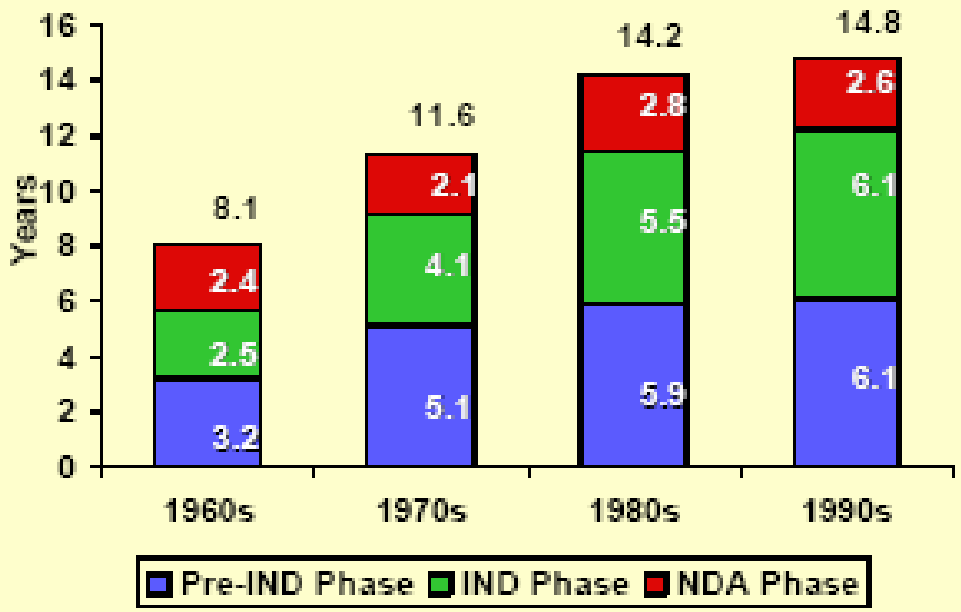
R&D Cycle Times are Increasing



Source: Joseph A. DiMasi, "New Drug Development: Cost, Risk, and Complexity," Drug Information Journal, May 1995. (From R&D Directions, 1996)

Development times for vaccines are the same or longer

From Dr. Jill G Hackell
AVP Scientific Affairs and Res Strategy

Adenovirus

MORPHOLOGY

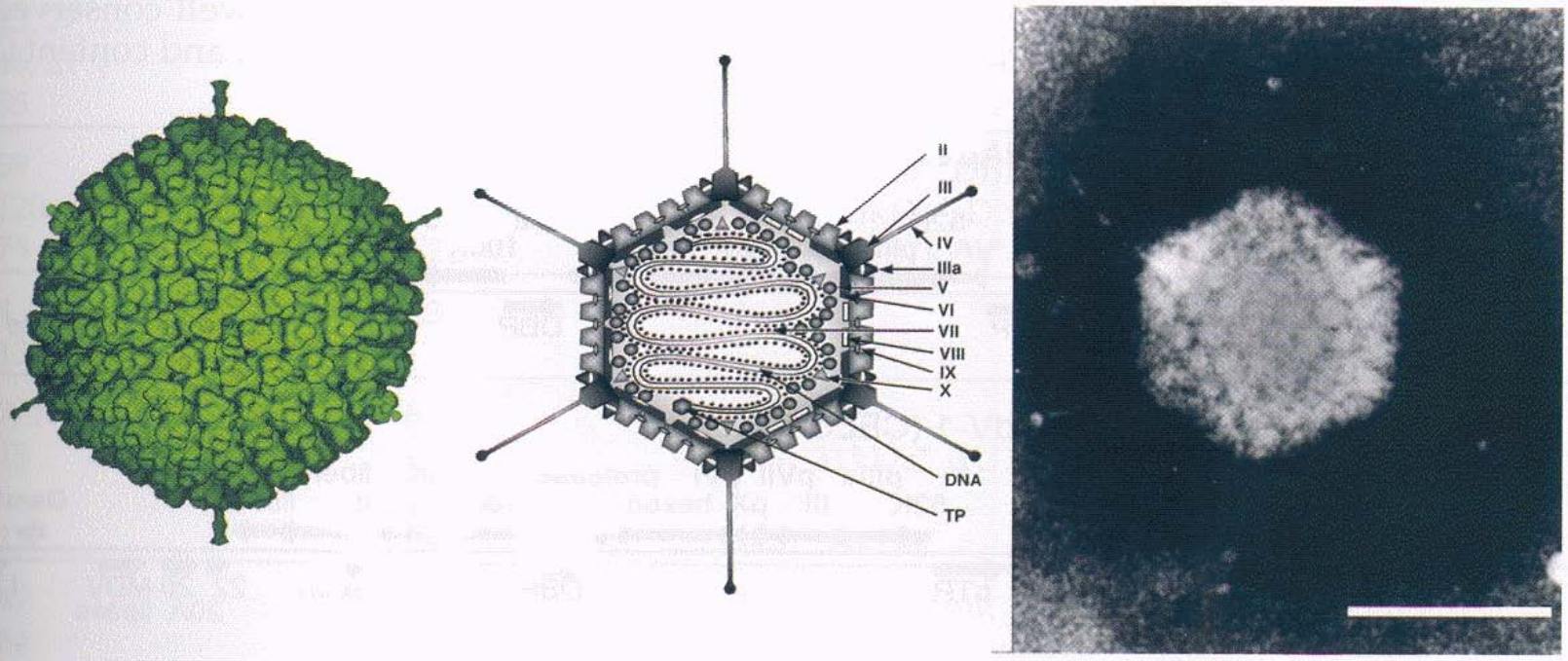
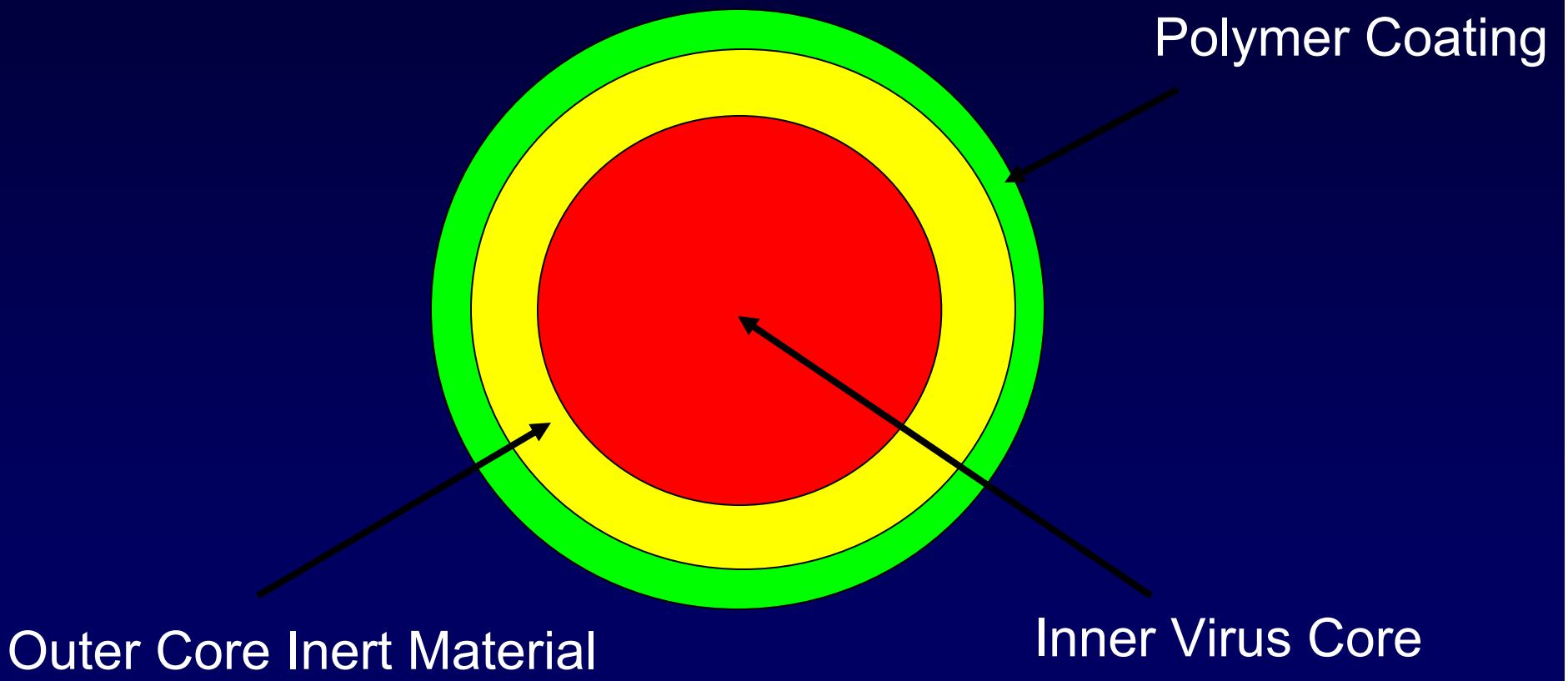


Figure 1: (Left) Cryo-electron reconstruction of a particle of an isolate of Human adenovirus 2 (Stewart et al. (1991). *Cell*, **67**:145-154). (Center) Stylized section of a mastadenovirus particle. For the description of the capsid (II, III, IIIa, IV, VI, VIII, IX) and core proteins (V, VII, X, TP), see text. As the structure of the nucleoprotein core has not been established, the polypeptides associated with the DNA are shown in hypothetical locations. (Adapted from Stewart, P.L. and Burnett, R.M. (1993). *Jpn. J. Appl. Phys.*, **32**, 1342-1347). (Right) Negative contrast electron micrograph of a particle of an isolate of Human adenovirus 2 (Valentine, R.C. and Pereira, H.G. (1965). *J. Mol. Biol.*, **13**, 13-20). The bar represents 100 nm.

Fauquet, CM et al, eds. Virus Taxonomy, p. 213

Adenovirus Vaccine



Pre-Phase 1 Seroprevalence Study

Objective:

Serotype 4 and type 7 seroprevalence among 91W's

Results:

99 91W blood donors tested

Adv 4 (+)	Adv 7 (+)	69%
Adv 4 (-)	Adv 7 (+)	9%
Adv 4 (+)	Adv 7 (-)	20%
Adv 4 (-)	Adv 7 (-)	2%
Adv 4 seropositive		89%
Adv 7 seroposivive		78%

Adenovirus 4 and 7 Seroprevalence

	No. Subjects	Adv 4(+)	Adv 7(+)
Pre-induction* 1998	303	34%	27%
Post-BT 2004	407	82%	82%

**Ludwig, et al JID 1998;178:1776-8*

Results: Safety (AE)

Adverse Events	Vaccine (N=30)	Placebo (N=28)
Nasal Congestion	10 (33.3%)	16 (57.1%)
Cough	10 (33.3%)	10 (35.7%)
Sore throat	8 (26.7%)	8 (28.6%)
Headache	6 (20.0%)	6 (21.4%)
Fever	2 (6.7%)	6 (21.4%)
Arthralgia	4 (13.3%)	0 (0.0%)
Nausea	4 (13.3%)	6 (21.4%)
Rhinorrhea	1 (3.3%)	3 (10.7%)
Wheezing	1 (3.3%)	3 (10.7%)
Pneumonia	1 (3.3%)	3 (10.7%)
Sinusitis	3 (10.0%)	2 (7.1%)
Abdominal pain	5 (16.7%)	1 (3.6%)
Diarrhea	4 (13.3%)	2 (7.1%)

Viral Shedding

Table 9.1. Adenovirus Isolation from Fecal Specimen by Treatment Group and Pre-immunization Antibody Status Over Time – All Treated Subjects

	VACCINE			PLACEBO		
Type 4	**Antibody (-)**	**Antibody (+)**	**Total**	**Antibody (-)**	**Antibody (+)**	**Total**
Stool Virus	**(+) / N**	**(+) / N**	**(+) / N**	**(+) / N**	**(+) / N**	**(+) / N**
Day 0	0 / 11	0 / 19	0 / 30	0 / 10	0 / 18	0 / 28
Day 7	7 / 11	0 / 18	7 / 29	1 / 10	0 / 17	1 / 27
Day 14	6 / 11	0 / 18	6 / 29	1 / 9	0 / 17	1 / 26
Day 21	1 / 11	0 / 18	1 / 29	0 / 9	0 / 17	0 / 26
Day 28	0 / 11	0 / 18	0 / 29	0 / 9	0 / 16	0 / 25
Day 56	0 / 11	0 / 18	0 / 29	0 / 9	0 / 16	0 / 25
Overall*	8 / 11	0 / 19	8 / 30	2 / 10	0 / 18	2 / 28
Type 7	**Antibody (-)**	**Antibody (+)**	**Total**	**Antibody (-)**	**Antibody (+)**	**Total**
Stool Virus	**(+) / N**	**(+) / N**	**(+) / N**	**(+) / N**	**(+) / N**	**(+) / N**
Day 0	0 / 17	0 / 13	0 / 30	0 / 14	0 / 14	0 / 28
Day 7	10 / 16	6 / 13	16 / 29	0 / 14	0 / 13	0 / 27
Day 14	5 / 16	3 / 13	8 / 29	0 / 13	0 / 13	0 / 26
Day 21	0 / 16	0 / 13	0 / 29	0 / 13	0 / 13	0 / 26
Day 28	0 / 16	0 / 13	0 / 29	0 / 13	0 / 12	0 / 25
Day 56	0 / 16	0 / 13	0 / 29	0 / 13	0 / 12	0 / 25
Overall*	12 / 17	6 / 13	18 / 30	0 / 14	0 / 14	0 / 28

*Subject who tested positive at multiple time points were only counted once for overall.